0022-202X/81/7603-0151$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 76:151–157, 1981

Vol. 76, No. 3
Printed in U.S.A.

REPORTS

Scanning and Transmission Electron Microscope Study on the Terminal Blood Vessels of the Rat Skin

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The microvasculature in the subepidermal layer of the rat foot was examined by scanning electron microscopy of corrosion casts and by transmission electron microscopy of serial ultrathin sections.

Three-dimensional observation of the casts demonstrated that, in the walking pads (pressure areas) with a thick epidermis, terminal vessels formed tortuous capillary loops that penetrated vertically into well-developed dermal papillae. In other regions of the foot (nonpressure areas) with a thin epidermis, terminal vessels formed a horizontally arranged capillary network and each capillary of the network fitted into a shallow groove along the dermal epidermal boundary base.

These differences in the pattern of vascular distribution might be of significance from the view point of the blood flow; in pressure areas, the vertically arranged, tortuous vessels might allow vertical mobility of the skin without injury to them and might keep the blood flow normal against the force of compression.

In thin sections, however, capillaries of both the loops and networks were similar in spite of the differences in the vascular distribution and the architecture of the epidermal-dermal junction. Vessels located in close proximity to the epidermis exhibited endothelial fenestrations along their proximal margins. In vessels away from the epidermis, on the contrary, fenestrations were not apparent.

Since endothelial fenestration is an anatomical property related to rapid material exchange, it may be concluded that the pattern of distribution and the fine structure of subepidermal capillaries adjusts to the metabolic needs of the epidermis.

The structure of the microvasculature in the subepidermal layer has been widely investigated both at the light microscopic and ultrastructural level, because of its possible functional role not only in the nutrition of the epidermis but also in the regulation of body temperature and blood pressure. No general agreement, however, exists on the ultrastructural features of capillaries in the dermal papillae. For example, there are contradictions as to the presence of endothelial fenestrations; Odland [1] and Braverman and Yen [2] reported that the capillary loops in the dermal papillae of human forearm flexor and elbow skin had a thick endothelium without fenestrations. On the other hand, McLeod [3] Seifert and Klingmuller [4] and Takada and Hattori [5] observed endothelial fenestrations in dermal capillaries of the scalp, finger, chest and in other regions of man and on the skins of several laboratory animals. So, Braverman and Yen [2] suggested that the subepidermal layer for most of human skin was provided with nonfenestrated capillary loops. They also suggested that some skin regions such as fingertips and heels, which had highly developed rete ridges, might have capillary loops with a different organization and ultrastracture: endothelial fenestrations.

The purpose of the present study is to elucidate the fine structure of the terminal blood vessels in the subepidermal layer both by scanning and transmission electron microscopy. Particular attention was focused on the problem of whether the ultrastructural features of the capillaries were variable depending on the distribution pattern of the vascular bed and/or the architecture of the dermal papillae.

MATERIALS AND METHODS

Adult albino rats (Wistar strain, weighing 150–200 gm) were used in this study. All the animals were anesthetized by intraperitoneal injection of Nembutal (30 mg/kg) before tissues were processed for subsequent electron microscopy.

The sole skin of rats was chosen for the present work, since it was shown from the preliminary scanning electron microscopy of corrosion casts and light microscope study that this region exhibited a variety of vascular patterns and thicknesses of the epidermis.

For scanning electron microscopy, Mercox (Dainihon Ink Co. Ltd., Tokyo, Japan, principally composed of acrylate monomer) was injected through the femoral artery, until the femoral vein was filled with the injected resin. After the resin was polymerized for 2 to 3 hr, the feet were removed and macerated completely in 20% aqueous potassium hydroxide solution for 3 to 4 days [6]. Corrosion casts of the feet thus prepared were washed thoroughly in running water, air-dried, coated with carbon and gold, and examined in a JEOL JSM-S1 scanning electron microscope.

For transmission electron microscopy, the skin of the feet was perfused through the left ventricle with half-strength Karnovsky's fixative [7] for 3 to 5 min. Small pieces of the skin were removed from several regions of the feet: walking pads, and arches of the toe and the planta. Specimens were immersed for 2 hr in the same fixative, rinsed briefly in the same buffer solution and postfixed in 1% osmium tetroxide for 2 hr. After fixation, materials were immersed in 4% aqueous uranyl acetate for 40 min for block staining. Materials were, then, dehydrated in graded ethanols and embedded in epoxy resin. Thin sections were made with glass knives on a Porter-Blum MT-1 microtome and stained with lead tartrate. Serial sections from certain regions of the skin were also made to examine the distribution range of endothelial fenestrations. All the sections were examined in an JEOL 100B electron microscope.

RESULTS

Scanning Electron Microscopy

By using the corrosion casting method, the entire architecture of the blood vascular bed of the rat foot could be easily demonstrated.

In the walking pads, especially in the toe pads, the blood vessels of the upper dermis showed the so-called "Candelabra" pattern; the vessels arising from the terminal arterioles were further divided into several capillary loops at the subpapillary layer. Superficial vessels formed the vertically standing tortuous capillary loops with ascending and descending limbs which were sometimes interconnected by collateral capillary

Manuscript received January 14, 1980; accepted for publication August 13, 1980.

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branches (Fig 1*a*). In the interdigital walking pads, the loops were arranged in rows and some neighboring loops were connected with each other at their apices (Fig 1*b*).

In the other parts of the foot, the vascular bed showed a quite different arrangement from that in the walking pads. Terminal vessels here formed a small-meshed capillary network running parallel to the skin surface (Fig 2*a*). In spite of relatively high frequency of branching of the superficial capillary network, a small number of capillaries were observed to originate from the terminal arterioles (Fig 2*b*). A coarse network of collecting vessels was observed beneath the capillary network (Fig 2*a*); postcapillary venules arose from branching points of the capillary network and passed deeply into the dermis where they drained into collecting venules and successively into veins (Fig 2*c*). Because arterioles lost their individual character before reaching the superficial network while venules arose below the plane in which this net was spread, the superficial network consisted exclusively of capillaries (Fig 2*abc*).

The casts of dermal vessels showed distinct differences between arteries and veins; as a rule, the arterioles were of smaller diameter compared with the venules, followed a straighter course and branched with acute angles. In contrast to the arterioles, the venules were of larger diameter, exhibited a more tortuous course and joined some of their tributaries at right angles (Fig 1*a*, 2*bc*).

Transmission Electron Microscopy

1. Capillary loops in the walking pads: In sections cut vertical to the skin surface, the epidermis of the walking pads was very thick and the dermis appeared to be supplied with elaborate blood vessels in its subepidermal layer. Long capillary loops penetrated into the dermal papillae between epidermal ridges (Fig 3–6). From observations of serial sections, it was found that each capillary loop in a dermal papilla showed fenestrated and nonfenestrated regions depending upon its spatial relation to the epidermis (Fig 8*abc*). This diversity in endothelial structure was often found repeated in the course of a single loop within a papilla.

When the capillaries were distant from the epidermis, they were nonfenestrated and measured 4–7 μm in luminal diameter. The endothelium of nonfenestrated vessels was 2 to 3 times as thick as the diameter of the cytoplasmic vesicles even in the attenuated portions (Fig 8*ab*). In such regions, 2 or 3 communicated vesicles were occasionally observed to fuse with both luminal and basal plasma membranes leaving diaphragms, thus presumably forming a channel across the endothelium (Fig 10). Attenuated portions of the nonfenestrated endothelial tube were usually invested by pericytes and their processes (Fig 8*a*).

In serial sections, it was shown that the endothelium became increasingly attenuated and fenestrated, as the capillary loop approached the epidermis (Fig 8*abc*). Simultaneously, the luminal diameter of the capillaries became wider to 7–11 μm. The sites of endothelial fenestrations were devoid of pericytic processes (Fig 8*abc*). Very thin processes of the veil cells (fibroblasts), but never pericytic processes, sometimes intervened between the endothelium and the epidermal cells (fig 3–6). These fenestrated segments of the loops were found not only at the apices but also at other segments of the loops where they were adjacent to the epidermis (Fig 4,5,6).

The fenestrae occurred only in extremely attenuated portions of the capillary endothelium, where the wall thickness was less than the diameter of the cytoplasmic vesicles (Fig 9). These vesicles were 50–70 nm in diameter, often found close or fused to the endothelial plasma membrane, and were evenly distributed in the capillary endothelium. The diameter of the fenestrae was 50–60 nm, and each of them was always closed by a thin diaphragm with a central dot (Fig 9). The fenestrae usually occurred in groups and faced toward the epidermis (fig 4,5,6). On the contrary, the perinuclear thick portion of the capillary endothelium was situated on the side opposite to the epidermis. Most of the cell organelles such as mitochondria, smooth and rough endoplasmic reticulum, the Golgi complex, multivesicular bodies and cytoplasmic filaments were seen mainly in proximity to the tapered ends of a flattened nucleus (Fig 11). Rod-shaped, tubulated, Weibel-Palade bodies [8] were also observed in the perinuclear region.

As the capilliary loops left the dermal papillae, they joined with neighboring and/or interconnecting capillaries and increased gradually in the wall thickness and the luminal diameter (Fig 3). After the transition of capillaries to postcapillary venules [9] the endothelial fenestrations were no longer observed.

In the walking pads, large dermal papillae which contained both the Meissner's corpuscles and capillary loops were frequently observed in addition to the narrower papillae which contained capillary loops alone. The capillary loops in these large papillae were situated in spaces between the corpuscles and the epidermis. Such loops were essentially identical in ultrastructure as those in the narrow papillae, except that they were larger and more numerous; the capillaries were dilated and fenestrated when they were close to the epidermis, and the attenuated and fenestrated portion of the capillary endothelium consistently faced toward the epidermis (Fig 6).

2. Capillary networks: The skin of the foot except for the walking pads was provided with a dense horizontal capillary network in the subepidermal layer, as observed by scanning electron microscopy. In thin and thick sections, however, the morphological appearance of the vascular bed in the dermis varied considerably depending on the cutting direction. When sectioned perpendicular to the skin surface, the skin of toe and plantar arches showed a thin epidermis and a very limited distribution of the vascular bed in the dermis. In the subepidermal layer, both longitudinal and transverse sections of capillaries were observed lying beneath the shallow grooves of the epidermal base (Fig 7). Thus the architecture of the epidermal-dermal junction in these regions was not like the dermal papillae as found in the walking pads, but instead formed a network of low dermal ridges in which the capillary network fitted.

Each capillary fitted into a low dermal ridge and showed both fenestrated and nonfenestrated segments, as seen in the capillary loop of the dermal papilla. The capillaries located in close proximity to the epidermis were observed to have many fenestrations in the attenuated portions of the endothelium. These fenestrae were occurred in groups and faced toward the epidermis (Fig 7). The capillaries apart from the epidermis did not show any endothelial fenestrations. From the study of serial sections, it appeared that the fenestrated segments of the network were shorter than those of the loops in the walking pads.

The endothelium contained a moderate number of cytoplasmic vesicles, which were consistently observed over the entire endothelium of the capillaries. Transendothelial channels consisting of communicated vesicles were also found in both fenestrated and nonfenestrated segments of the capillary network.

The endothelium of both the capillary loops and the networks were sealed by tight junctions between 2 adjacent cells. The number of fusion points between cell membranes was, in most cases, only one in the fenestrated segments, but 2 or 3 in the nonfenestrated segments (Fig 8*abc*, 11).

DISCUSSION

Three-dimensional architecture of the blood supply to the dermis in mammals, to our knowledges, has not been described so far, except for the reconstruction study of some capillary loops by serial thick sections [2].

The present study has clearly demonstrated differences in the three-dimensional vascular arrangement of the subepidermal layer between walking pads and the other regions of the rat foot. It is generally held that differences in vascular distribution correlate with differences in the epidermal thickness and in the architecture of the epidermal-dermal junction, etc.

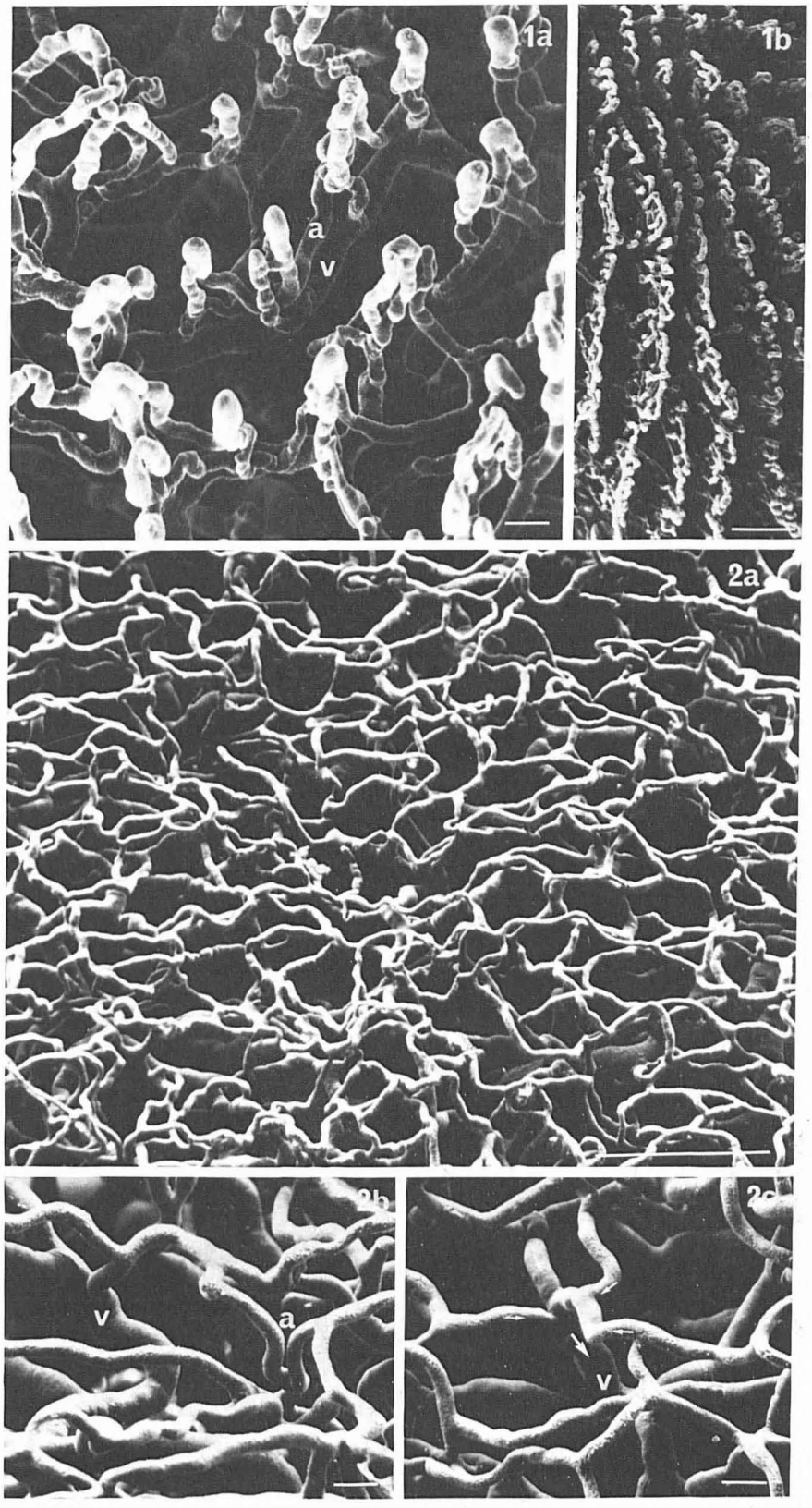


FIG 1–2. Survey views of the vascular corrosion casts of the skin of the rat foot. 1*a*. Capillary loops from the toe pad. Tortuous loops and their afferent (*a*) and draining (*v*) vessels form the so-called "Candelabra" pattern. Knotted portions of the loops must represent fenestrated segments, because it was determined from the study of thin sections that the capillaries of fenestrated segments were larger in luminal diameter than those of nonfenestrated segments. Grouped loops in the upper left corner are the loops in a large papilla which contains Meissner's corpuscles. *Bar* = 10 μm 1*b*. Loops from the interdigital walking pad. The loops are arranged in rows and some of the loops are connected at their apices. *Bar* = 100 μm 2*a*. Subepidermal capillary network from the toe arch. Large draining vessels with numerous tributaries and several small supplying vessels are seen in the back ground. *Bar* = 100 μm 2*b*. Network from the plantar arch. Supplying vessels (*a*) have a smaller diameter compared with draining vessels (*v*) and are characterized by a straighter course and, in most cases, forked ramifications. *Bar* = 10 μm 2*c*. Network from the plantar arch. Area of venule formation. Arrows indicate the direction of blood flow. Draining vessels (*v*) exhibit a tortuous course, join some tributaries at right angles, and increase their diameter with every confluence. *Bar* = 10 μm.

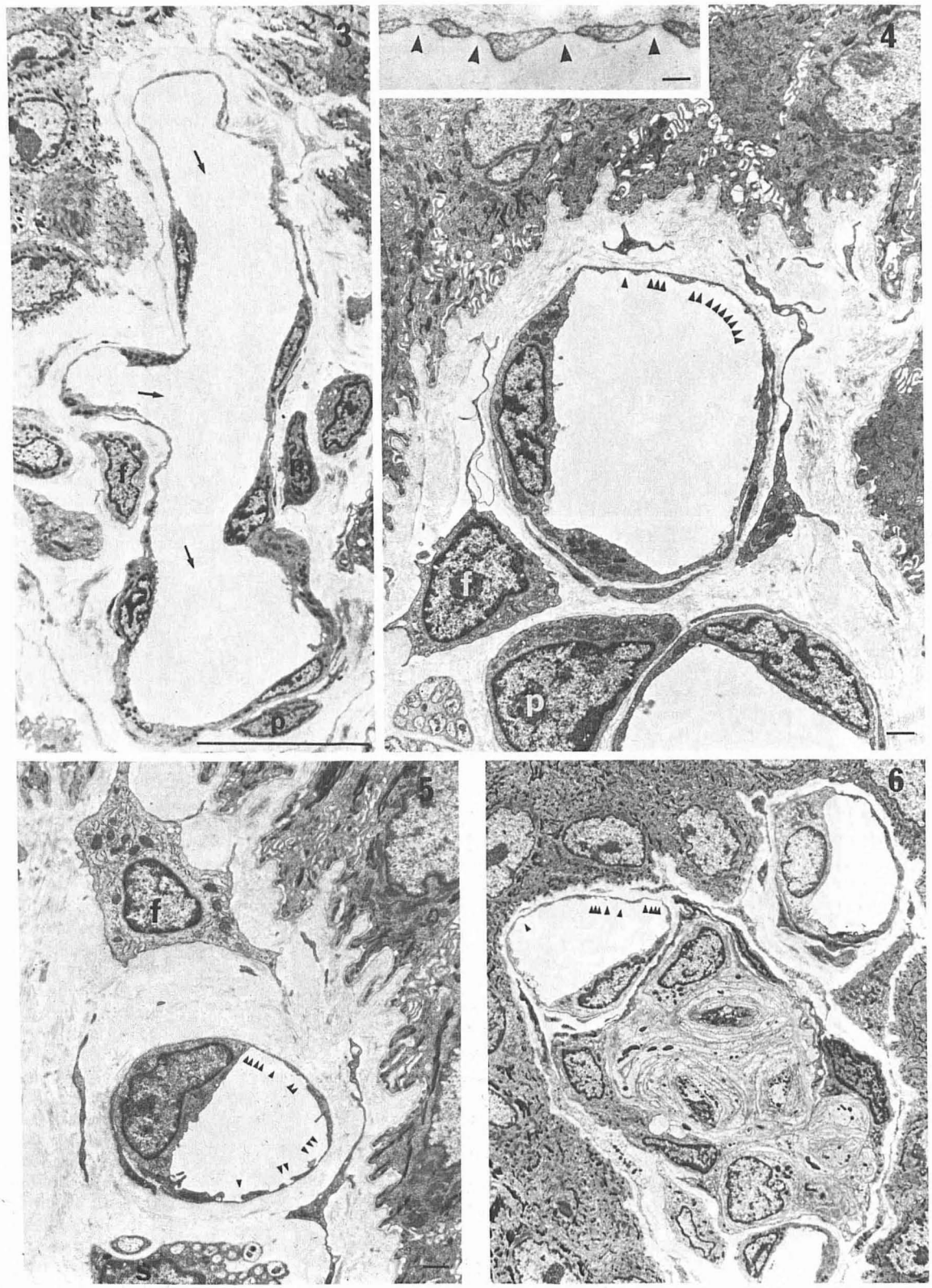


FIG 3. Longitudinal section of a descending limb of a capillary loop. *Arrows* indicate the direction of blood flow. A gradual increase of the wall thickness and of the luminal diameter is observed in the transition from capillary to postcapillary venule [9]. Fenestrae are no longer observed after the capillary has left the dermal papilla. Ascending limbs, which form a direct continuation of the terminal arterioles, have a relatively thick endothelium in comparison to a smaller luminal diameter of 4–7 μm, until they approach the epidermis. Cross section of this segment is shown in Fig 6. *p*:pericyte, *f*:veil cell. *Bar* = 10 μm.

FIG 4. Cross sectional view of an apex of a capillary loop. Attenuated and fenestrated portion of the endothelium (*arrowheads*) faces toward the epidermis. Note the positions of a pericyte (*p*) and a veil cell (*f*); they keep away from the fenestrated portion of the endothelium. *Bar* = 1 μm (=0.1 μm, *Inset*).

FIG 5. Cross section of an ascending limb of a capillary loop. The endothelium is fenestrated (*arrowheads*) both at the apex and along the course of the loop where it borders upon the epidermis. *f*:veil cell, *s*:Schwann cell. *Bar* = 1 μm.

FIG 6. Section of the toe pad. A large papilla contains Meissner's corpuscles and capillary loops. The capillary in the base of the papilla has a thick, nonfenestrated endothelium and a small luminal diameter of about 4 μm, while the capillaries between the epidermis and the corpuscles have a fenestrated endothelium and large luminal diameter of 7 to 11 μm. The fenestrae (*arrowheads*) occur only at the epidermal side of the capillaries. *Bar* = 1 μm.

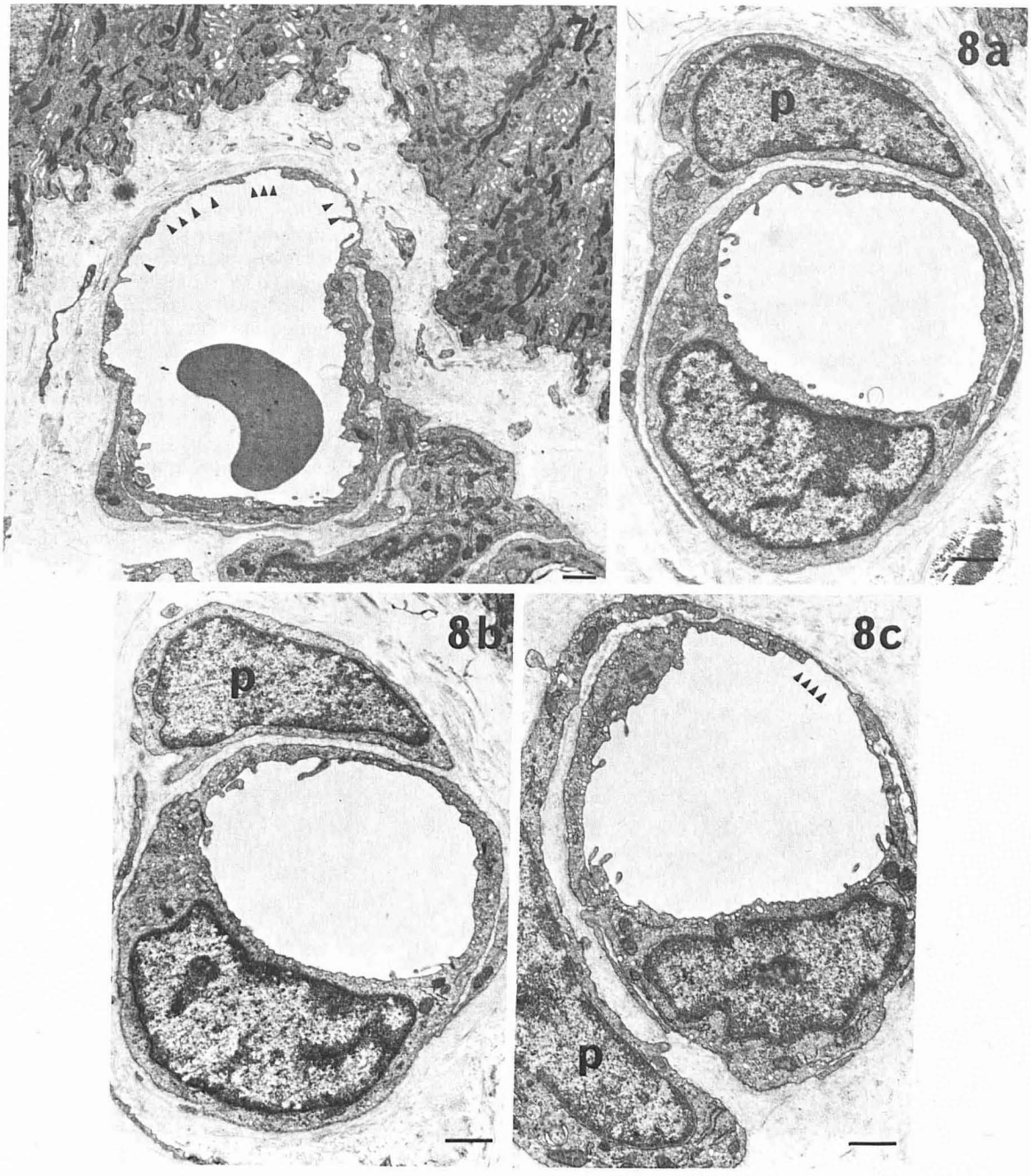
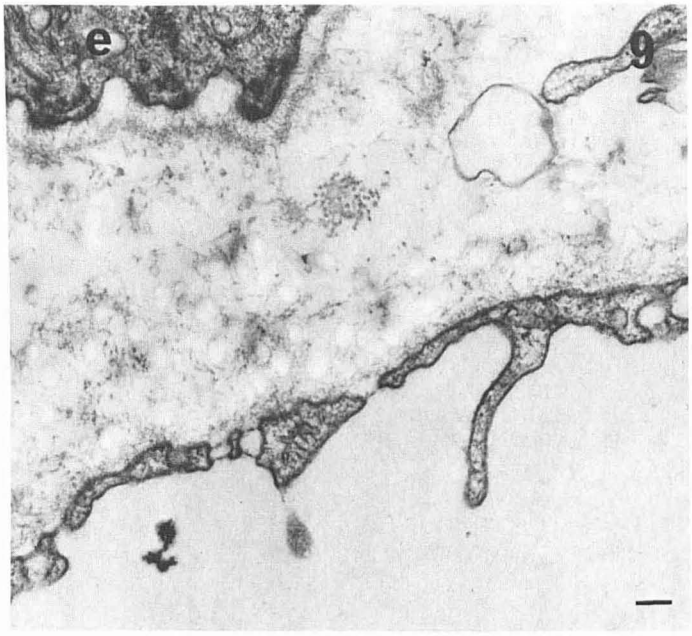


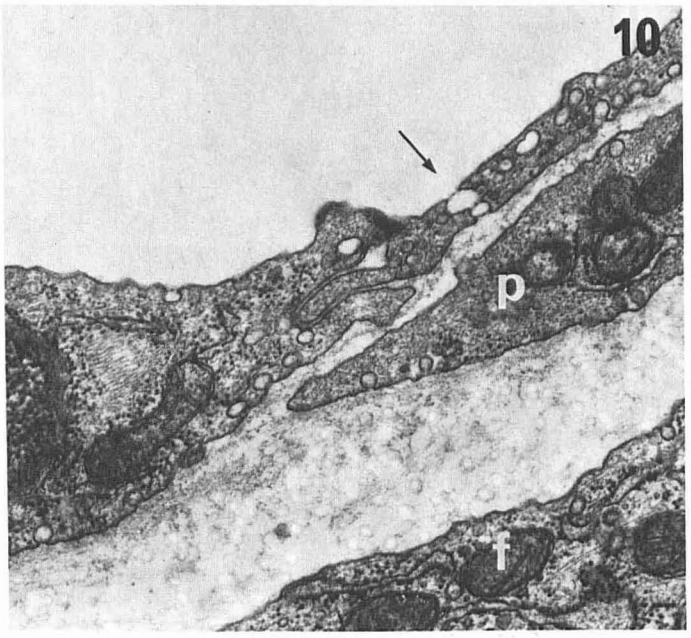
FIG 7. Cross sectional view of a network capillary from the plantar arch. The base of the epidermis is caved shallowly in accordance with the capillary. Endothelial fenestrae (*arrowheads*) face toward the epidermis without the interposition of pericytes and veil cells. *Bar* = 1 μm.

FIG 8. *a,b,c*, Near-serial sections of a capillary loop cut oblique to the skin surface. The micrographs show a diversion of the capillary from nonfenestrated to fenestrated type. In the nonfenestrated segment, a pericyte(p) invests most of the endothelial tube as if to reinforce the attenuated portion. The cell body and cytoplasmic processes of the pericyte are absent from attenuated portion of the endothelium, where fenestrae (*arrowheads*)) occur. *Bar* = 1 μm.

[10,11]. It is also possible that the action of exogenous force on the skin surface may be related to the variation in vascular arrangement in the dermis and may be of great significance in terms of the dynamics of the blood flow. Vertically arranged, tortuous vessels might allow vertical mobility of the skin without injury to the vessels and might keep the blood flow normal in spite of external pressures applied vertical to the skin surface. This seems to be the case in the skin of the walking pads, where the so-called "Candelabra" patterns of blood vessels (including the capillary loops) are arranged perpendicular to the skin surface. On the contrary, horizontally arranged capillary networks, as found in the other regions of the foot, could prevent the lumen from collapsing only against weak external pressures.

In the present study, it was demonstrated that most of the skin of the rat foot was provided with a horizontal capillary network in its subepidermal layer, except for the walking pad skin which was provided with a vertical system of the capillary loops. This finding, coupled with the recent reports that the subepidermal capillaries are organized in network pattern in lower vertebrates [12,13], leads us to the speculation that the horizontal capillary networks are the original vascular arrangement in the subepidermal layer, and that the capillary loops





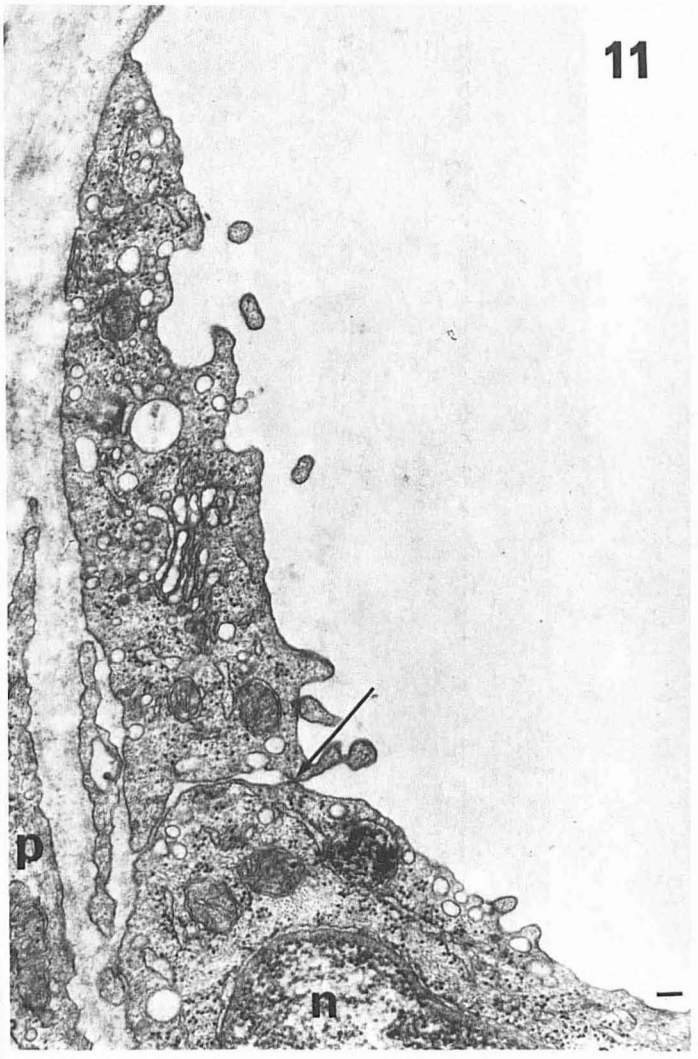


FIG 9. A fenestrated portion of a capillary endothelium. Apparent transendothelial channels formed by single cytoplasmic vesicles are also demonstrated. Note that the thickness of the endothelium is less than the diameter of the cytoplasmic vesicles. *e*:epidermis. *Bar* = 0.1 μm.

FIG 10. Apparent transendothelial channel (*arrow*) of a nonfenestrated capillary endothelium. Each fusion point of the vesicles is closed by a diaphragm which is similar to that of fenestrae. *p*:pericyte, *f*:veil cell. *Bar* = 0.1 μm.

FIG 11. Perinuclear zone of an endothelial cell of a fenestrated capillary. Most of the mitochondria, rough and smooth endoplasmic reticulum, Golgi complex, vesicles and vacuoles, and filaments of both 6 nm and 10 nm diameters are found near the nucleus(*n*). Intercellular junction of the endothelium is a tight junction with only one fusion point (*arrow*). *p*:pericyte. *Bar* = 0.1 μm.

are rather a specialized one. Althought it has been assumed that the human skin is provided with capillary loops [10,11], the present study may suggest that considerable area of the human skin is also provided with horizontal capillary networks in the subepidermal layer. This suggestion might be supported from some clinical lesions of the human skin. For instance, telangiectases, characterized by dilated superficial blood vessels, usually show reticular arrangement.

It has been assumed that the primary purpose of the skin circulation is not metabolic exchange to maintain tissue structure, but rather exchange of heat and regulation of blood pressure, because the vascularity and the blood flow is developed beyond the degree necessary for nutrition of the comparatively thin epidermis and the small number of adnexal structures present in the digit [10]. Thus, little attention has been paid to the material exchange between the blood vessels and the epidermis. Fenestrated capillaries have been reported only where dermal papillae are "well developed" [3,5], none or few if any, exist in "poorly developed" papillae, namely in the capillaries beneath the thin epidermis [1,2,5]. Based on the present study by scanning and transmission electron microscopy, however, it was revealed that capillaries organized into loops and networks were similar in fine structure without regard to the differences in the vascular distribution and the architec-

ture of the epidermal-dermal junction. That is, they consistently exhibited fenestrated segments along their course. Fenestrated portions of the capillaries must represent areas with high permeability for water and electrolytes, and large molecules as well [14,15]. The fact that the capillaries in the subepidermal layer are fenestrated when located close to the epidermis, and that the fenestrations ordinarily face toward the epidermis may imply the adjustment of the subepidermal capillaries to the high metabolic needs of the epidermal cells.

In the present study, it was also demonstrated that cytoplasmic vesicles were aligned across endothelial cell processes, thereby suggesting the formation of transendothelial channels. Such transendothelial channels have been described by others in some fenestrated [14,16], and continuous capillaries [15,17]. Cytoplasmic vesicles are found over the entire endothelium of the capillary loops and networks, and they are believed to be involved in material transport across the endothelium [18,19]. Thus, these channels, although relatively infrequent, in the vessels investigated here are probably additional pathways for transendothelial transport, particularly in nonfenestrated capillaries.

Odland [1], and Braverman and Yen [2], demonstrated collapsed vessels in their illustrations. In their opinions, the ultrastructure of dermal capillaries in the human skin were distinct from those in other vertebrates and was characterized by a thick endothelium with a large number of 10 nm cytoplasmic filaments [1] and by homogenous or multilaminated basal lamina around the endothelial tube [2]. In the present study of rat skin, dermal capillaries tended to exist in a nearly collapsed state and were observed to have thick endothelium and partially multilaminated basal lamina, only when the skin was unsuccessfully perfused by the fixative. It is conceivable that the variation in ultrastructural appearance of dermal capillaries depends not so much on the differences in species but rather on the differences in the fixation procedure. The effects of biopsy and successive immersion fixation on the fine structure of dermal blood vessels will be described in another paper.

The author wishes to acknowledge the helpful advices to Prof. T. Yamamoto.

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